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Note

Potentiometric Study of Holmium Complexes with EDTMP

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• holmium
• EDTMP (ethylenediamine-*N*,*N*,*N'*,*N'*-tetra(methylenephosphonate)
• potentiometry

Within the study of ^{166}Ho-complexes as potential radiopharmaceutical agents, stability constants of those with EDTMP (ethylenediamine-*N*,*N*,*N´*,*N´*-tetra(methylenephosphonate)) were calculated by the computer program LETAGROP Etitr using data from potentiometric titrations measured at 25 °C in 0.1 M NaCl. The following values were obtained for stability constants of Ho-EDTMP complexes $Ho(OH)L^{6-}$, HoL^{5-}, $HHoL^{4-}$, H_2HoL^{3-}, H_3HoL^{2-} and H_4HoL^{-}: log β = 9.31, 20.22, 28.14, 34.55, 39.89, 45.05, respectively. Diagram of the complex speciation dependence on pH shows that complex forms $HHoL^{4-}$and H_2HoL^{3-} are characteristic of the pH range 6–8, which is adequate for their application into the human body.

INTRODUCTION

Owing to its physical properties (high-energetic β radiation and short half-life), radionuclide ^{166}Ho is a very attractive isotope for radiotherapy. Complexes of ^{166}Ho with phosphonates such as DOTMP (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylenephosphonate)),[1] APDDMP (*N*,*N*-dimethylenephosphonate-1-hydroxy-4-aminopropilydenediphosphonate),[2] EDTPM (ethylenediamine-*N*,*N*,*N'*,*N'*-tetra(methylenephosphonate))[3,4] have been investigated as possible radiotherapeuticals for pain relief and treatment of bone metastases. The complexes of bisphosphonates, MDP (methylenediphosphonate) and HEDP (1-hydroxyethylenediphosphonate) have been applied in bone-imaging radiopharmaceuticals.[5]

The yields of ligand complexation with metal are usually determined by thin-layer chromatography and HPLC. In the case of the EDTMP-Ho system, complexation was studied by the TLC method.[6]

The aim of the present study is to complete the information about Ho-EDTMP complexes by determining the stability constants obtained from potentiometric titrations.

EXPERIMENTAL

Chemicals

Solutions of sodium hydroxide of concentrations in the range from 0.01 mol dm^{-3} to 0.09 mol dm^{-3} were used as titration agents. The solutions were prepared from the saturated stock solution of NaOH by the Sörensen method. Titration mixtures were prepared from stock solutions obtained by diluting a requisite amount of substance in an appropriate volume of 5×10^{-3} mol dm^{-3} EDTMP acid and 0.01 mol dm^{-3} holmium nitrate. EDTMP was prepared in the laboratory of the Department of Organic Chemistry, Natural Science Faculty of Charles University in Prague. Holmium nitrate was purchased from Aldrich Prague, Czech Republic. All other chemicals were made at Lachema Brno in the Czech Republic and were of analytical reagent grade purity.

Procedure

Experimental data for the calculation of constants were obtained by potentiometric titration of the required mixtures. An automatic titrator Radiometer RTS 822 with a glass electrode G 2040 and calomel electrode K 4040 was used

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for the measurement of potentiometric data. The glass electrode was calibrated using standard buffer solutions (saturated solution of potassium hydrogen tartarate pH = 3.557, 0.05 mol dm^{-3} potassium hydrogen phthalate pH = 4.008, 0.025 mol dm^{-3} phosphate buffer pH = 6.865, 0.0087 mol dm^{-3} potassium dihydrogen phosphate / 0.03 mol dm^{-3} sodium hydrogen phosphate pH = 7.413, 0.01 mol dm^{-3} sodium tetraborate pH = 9.180, saturated solution of calcium hydroxide pH = 12.454; temperature 25 °C). The accuracy of the calibration procedure was successfully verified by measuring the pH values of the set of acid solutions (standardized by titration). Standard buffer solutions used for the calibration helped extend the applicable pH range of further experiments. Such calibration reduced the deviation of the glass electrode response.

All titrations were performed at a temperature of 25 °C at constant ionic strength of 0.1 mol dm^{-3} NaCl in the inert atmosphere of nitrogen to avoid CO_2 absorption by the basic solutions. The pH range of the titrations was 2–10. It was found that the kinetics of hydroxide reaction with the ligand was fast enough and therefore the pH values were registered one minute after addition of the titration agent. EDTMP acid was titrated in a concentration range from 5×10^{-4} mol dm^{-3} to 2×10^{-3} mol dm^{-3}. The titrations in the presence of holmium nitrate were carried out at the EDTMP:Ho molar ratio 1:1 and 2:1 for the ligand concentrations 2×10^{-3} mol dm^{-3}, 1×10^{-3} mol dm^{-3} and 5×10^{-4} mol dm^{-3}. It must be pointed out that no precipitation was formed in the pH region pH = 2–11.

RESULTS AND DISCUSSION

The H^+–L^{8-} system can be characterized by equations (L^{8-} is the EDTMP anion):

$$n\mathrm{H}^+ + \mathrm{L}^{8-} \leftrightarrow \mathrm{H}_n\mathrm{L}^{(8-n)-} \qquad (1)$$

where Eq. (1) characterizes the protonation of EDTMP, n is the protonation degree.

Reaction (1) and the following reaction (2) can be expected in the H^+–Ho^{3+}–L^{8-} system:

$$\mathrm{Ho} + n\mathrm{H}^+ + \mathrm{L}^{8-} \leftrightarrow \mathrm{H}_n\mathrm{HoL}^{(8-3-n)-} \qquad (2)$$

where Eq. (2) describes the holmium complex formation.

Reactions expressing the protonation of EDTMP and the complex formation allow defining the protonation constant:

$$\beta = \frac{[\mathrm{H}_n\mathrm{L}^{(8-n)-}]}{[\mathrm{L}^{8-}]\cdot[\mathrm{H}^+]^n} \qquad (3)$$

and the stability constants of complexes:

$$\beta = (\mathrm{H}_n\mathrm{HoL}^{(8-3-n)-}) = \frac{[\mathrm{H}_n\mathrm{HoL}^{(8-3-n)-}]}{[\mathrm{Ho}^{3+}]\cdot[\mathrm{L}^{8-}]\cdot[\mathrm{H}^+]^n} \qquad (4)$$

The stability constant of the HoL^{5-} complex is defined as:

$$K(\mathrm{HoL}^{8-}) = \frac{[\mathrm{HoL}^{5-}]}{[\mathrm{Ho}^{3+}]\cdot[\mathrm{L}^{8-}]} \qquad (5)$$

and the consecutive protonation constants of $H_nHoL^{(8-3-n)-}$ complexes can be described as:

$$K(\mathrm{H}_n\mathrm{HoL}^{(8-3-n)-}) = \frac{[\mathrm{H}_n\mathrm{HoL}^{(8-3-n)-}]}{[\mathrm{H}_{n-1}\mathrm{HoL}^{(8-4-n)-}]\cdot[H^+]} \qquad (6)$$

Computer program LTGW Etitr derived from the LETAGROP Etitr model, which was used for the evaluation of experimental data, helps solve the equation system ((1), (2) listed above) and calculate the stability constants defined by Eq. (3) and Eq. (4) by the non-linear regression method.

Protonation constants and complex stability constants were calculated by the program in a number of steps so as to make the calculated pH values approximate the values obtained by experimental measurement. Minimized function U can be expressed as:

$$U = \Sigma\ (\mathrm{pH}_{\mathrm{exp}} - \mathrm{pH}_{\mathrm{calcul}})^2 \qquad (7)$$

The value of $pK_w = 13.8$ was used in the calculation.

Program LTGW Etitr allows visualization of the calculated curve and the experimental values to assess whether the model with the U function minimum value really fits the experimental data.

Stability constant $K(HoL^{5-})$ is identical to $\beta(HoL^{5-})$. Consecutive protonation constants can be calculated from the equation:

$$\log K(\mathrm{H}_n\mathrm{HoL}^{(8-3-n)-}) = \log \beta(\mathrm{H}_n\mathrm{HoL}^{(8-3-n)-}) - \log \beta(\mathrm{H}_{n-1}\mathrm{HoL}^{(8-4-n)-}); \quad n > 0 \qquad (8)$$

The data in Table I show the protonation constants of EDTMP acid obtained by the above mentioned procedure.

The estimated protonation constants were used for determination of the stability constants of the Ho(III) complexes. The potentiometric study operates at the metal:ligand molar ratios of 1:1 and 1:2. The accuracy rapidly decreases for higher molar ratios due to the fact that the characters of the titration curve Ho-EDTMP and the titration curve of the ligand without metal are similar. The reciprocal ratios cannot be used because of the metal hydrolysis.

In the calculation, the existence of different protonated (or hydrolyzed) complexes from H_6HoL^+ to $Ho(OH)_2L^{7-}$ was assumed. The complex forms $Ho(OH)_2L^{7-}$, H_5HoL and H_6HoL^+ were not proven in the titration solution be-

TABLE I. Protonation constants (log β) of EDTMP acid

Form	log (β)				
HL^{7-}	10.3 ± 0.5	10.67 ± 0.01	9.638	12.01	12.99
H_2L^{6-}	20.0 ± 0.4	20.14 ± 0.01	17.330	21.03	22.77
H_3L^{5-}	27.9 ± 0.4	27.77 ± 0.01	23.594	28.45	30.71
H_4L^{4-}	34.4 ± 0.4	34.01 ± 0.01	28.636	34.33	37.13
H_5L^{3-}	39.6 ± 0.4	39.16 ± 0.01	31.501	39.10	42.30
H_6L^{2-}	42.2 ± 0.4	41.99 ± 0.01	32.624	41.92	45.32
Temp. / °C	25	37	25	25	25
Medium	0.1M NaCl	0.15M NaCl	0.15M NaCl	3M KNO_3	0.1M KNO_3
Lig. conc. / mol dm^{-3}	$5\times10^{-4}\div2\times10^{3}$	2×10^{-3}	1.5×10^{-3}	2×10^{-3}	2×10^{-3}
Ref.	This work	7	8	9	9

Note: Standard deviations were calculated at the significance level 3δ.

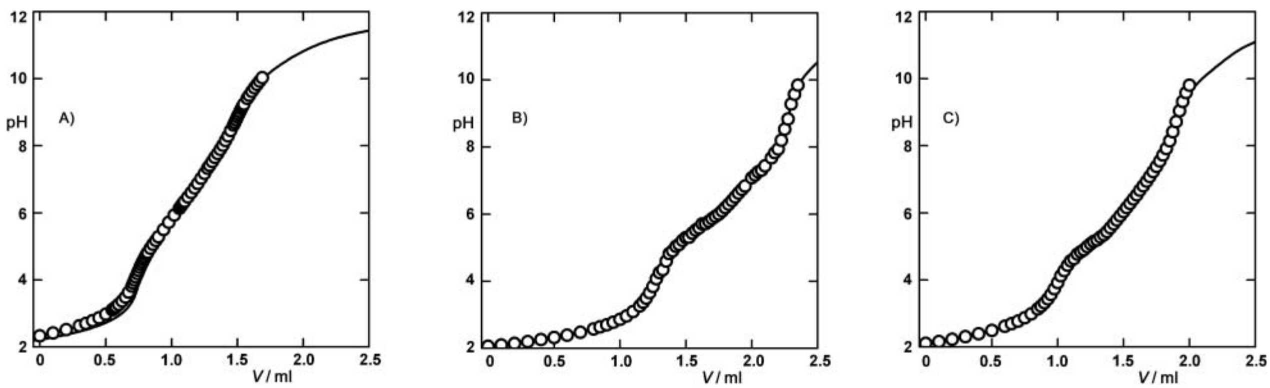

Figure 1. Titration curves of A) EDTMP; B) Ho-EDTMP molar ratio 1:1; C) Ho-EDTMP molar ratio 1:2. O experimental values; – calculated curve.

cause the minimum of U was reached for zero stability constants of these complexes (Figure 1 presents an example of the comparison between the calculated curve and experimental data).

The constants (summarized in Table II) calculated on the basis of the selected model made it possible to demonstrate the pH dependence of the fraction species in the Ho^{3+}-EDTMP system (Figure 2). Shares of holmium ions and holmium complexes in the total analytical concentration of holmium are defined as:

$$\delta = \frac{[\mathrm{H}_i\mathrm{HoL}]}{\sum_{i=-1}^{i-4}[\mathrm{H}_i\mathrm{HoL}]} \qquad (9)$$

The more protonated forms of complexes occur in the acidic pH range and the less protonated forms in the basic range. It can be assumed that the characteristic forms in neutral solutions ($5 < pH < 8$) are H_3HoL^{2-}, H_2HoL^{3-} a $HHoL^{4-}$. From the value of pH = 6.5, the ratio δ (Eq. 9) of the completely deprotonated form HoL^{5-} increases, and from the value pH = 8, the $Ho(OH)L^{6-}$ (written as $HoH_{-1}L^{6-}$) ion can be found in the solution. The deprotonated HoL^{5-} form reaches its maximum quantity in the solutions at pH = 9. This complex has a very large range of occurrence (pH~ 6–11). Because of the large number of complexes that can be formed, the ranges of their oc-

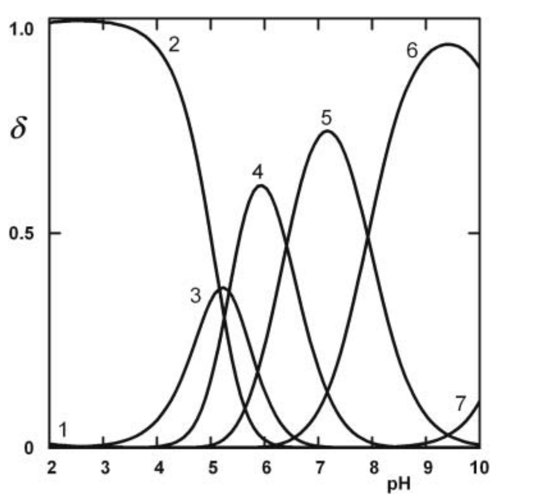

Figure 2. Fractions of the species containing holmium ion in the system Ho^{3+}-EDTMP. The curves were calculated for the constants given in Table I and Table II; 1: Ho^{3+}; 2: H_4HoL^{-}; 3: H_3HoL^{2-}; 4: H_2HoL^{3-}; 5: $HHoL^{4-}$; 6: HoL^{5-}; 7: $Ho(OH)L^{6-}$.

TABLE II. Stability constants (log β and log K) of Ho^{III} complexes with EDTMP

Complex form	log(β)	log K	log K
$HoH_{-1}L^{6-}$	9.3 ± 0.6		
HoL^{5-}	20.2 ± 0.5	20.2	13.32
$HHoL^{4-}$	28.1 ± 0.5	7.9	7.60
H_2HoL^{3-}	34.5 ± 0.5	6.4	6.33
H_3HoL^{2-}	39.9 ± 0.5	5.3	5.17
H_4HoL^{-}	45.0 ± 0.5	5.2	
Temperature / °C	25	25	37
Medium	0.1M NaCl	0.1M NaCl	0.15M NaCl
Ref.	This work	This work	7

Note: Standard deviations were calculated at the significance level 3δ.

currence overlap. For example, at pH = 5, the complex H_3HoL^{2-} maximum can be detected together with a high concentration of the H_2HoL^{3-} form; the ratio δ of this form reaches the maximum at pH = 6. The other form present at this pH value is H_4HoL^{-}. This form has the widest range of occurrence (besides HoL^{5-}) – from pH = 2 to 6.5.

The pH = 5 is specific to the complex protonated by one hydrogen ion because this complex starts to appear at this value and its concentration increases up to pH = 7 where the amount of this form reaches its maximum.

Comparison of our values of stability constants with that of Jarvis *et al.*[7] shows a great difference in $K(ML^{5-})$. It must be pointed out that the share of uncomplexed ion Ho^{3+} in the total concentration of Ho for pH = 5–9 studied in Ref. 7 is low, so the uncertainty in $K(HoL^{5-})$ is high. A more correct interpretation of Jarvis *et al.* data is log $K(ML^{5-}) \geq 13$.

On the other hand, there is very good agreement between the values of the ML^{5-} complex protonation constants calculated in Ref. 7 and in the present work and the calculated pH regions where these complexes prevail (Figure 2) are almost identical.

CONCLUSION

The dependence of holmium ion and complex shares on pH (Figure 2) are in agreement with the published data.[7] As our data were measured in a wide pH range (pH = 2 to 10), we obtained stability constants also for the complexes $Ho(OH)L^{6-}$ and H_4HoL^{-}.

The findings about the holmium ion complexes with EDTMP obtained from the potentiometric study extend the knowledge obtained by thin layer chromatography (TLC).[6] The TLC study showed that the optimal conditions for ^{166}Ho-EDTMP complexation are: the pH range from 6 to 8, the concentration ratio of ligand to holmium ion at least 250:1. The radiochemical yield of complexation is about 80 % under these conditions. The optimum pH range is also favorable for medical application. The forms of complexes that could be expected in the reaction solutions were determined on the basis of the potentiometric study (Figure 2). It is clear that the pH range from 6 to 8 is characteristic of the complex form H_2HoL^{3-} and $HHoL^{4-}$. Concentrations of these forms reach the maximum at pH ≈ 6 and pH ≈ 7, respectively. The ion complex HoL^{5-} could be also detected in the solution under the optimal conditions for radiolabeling.

The values of the stability constants of complexes can be useful in the simulation of their behavior in blood plasma.

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SAŽETAK

Potenciometrijska ispitivanja kompleksa holmija s EDTMP

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U okviru ispitivanja kompleksa ^{166}Ho kao potencijalnih radiofarmaceutika, određene su konstante stabilnosti holmijevih kompleksa s EDTMP (etilendiamin-*N*,*N*,*N'*,*N'*-tetra(metilenfosfonatom)) metodom potenciometrijske titracije. Titracije su izvedene kod 25 °C u 0.1 M NaCl, a pri obradi dobivenih rezultata rabljen je LETAGROP Etitr program. Dobivene su sljedeće vrijednosti za konstante stabilnosti Ho-EDTMP kompleksa: $Ho(OH)L^{6-}$, HoL^{5-}, $HHoL^{4-}$, H_2HoL^{3-}, H_3HoL^{2-} i H_4HoL^{-}; log β = 9,31; 20,22; 28,14; 34,55; 39,89 i 45,05. Grafički prikaz ovisnosti kompleksa o pH pokazuje da su u području pH između 6 i 8, koje je pogodno za humanu primjenu, prisutne $HHoL^{4-}$ i H_2HoL^{3-} kompleksne vrste.